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Arbuscular Mycorrhizae (AM) Spore Abundance and Edaphic Characteristics along a Successional Chronosequence

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ABSTRACT

Tropical soils are generally nutrient poor, even though they support high biodiversity and productivity. Most tropical plants are able to thrive in these soils because they form a mutualistic relationship with Arbuscular Mycorrhizae (AM), the latter of which increases nutrient absorption, and therefore the fitness of the host plant (though some species are more closely associated with AM than others). As a now-abandoned cattle pasture is allowed to passively regenerate (to re-grow forest without human intervention), edaphic properties (characteristics of the soil) and AM spore abundance may change in response to alterations in the plant community and structural changes (like increased leaf litter deposition). The present study examines spore abundance, nitrogen (N), phosphorus (P), potassium (K), pH, and bulk soil density for plots of various stages of regeneration from pasture (ages 0-45, and primary forest, N=15) in the Premontane Wet Forest near San Luis, Costa Rica. The data show substantial variation within and between sites in spore number and other soil characteristics. Regeneration age did not significantly affect AM spore number, macronutrient levels (NPK), or bulk density. pH was positively correlated with increasing site age, though this trend was not significant following the removal of two outliers. Spore abundance was not significantly correlated with N, P, K, pH, or bulk density. These findings suggest that AM spores may not be evenly distributed throughout the soil, instead exhibiting patchy distributions. It is possible that edaphic and AM trends with regeneration exist, but are confounded by other variables; however, it may also be that AM are so widespread throughout San Luis that AM infectivity is unlikely to direct forest regeneration.

INTRODUCTION

Deforestation rates are dramatically high in some parts of the world, such as in South America, which suffered a net forest loss of about 4.3 million hectares per year from 2000 to 2005 (Food and Agriculture Organization 2006). However, other tropical areas are actually experiencing net forest gains. Such is the case in Costa Rica, which from 2000 to 2005 increased its total forest cover by about 15,000 hectares (Food and Agriculture Organization 2006). As Costa Rica shifts from an agricultural and cattle-ranching economy to one of industry and tourism, farmland is being abandoned and allowed to regenerate into secondary forest. Though conservation efforts tend to focus on preserving primary ("undisturbed") forests, secondary forests are also an asset to conservation. Many ecologically important reserves in Costa Rica, such as Santa Rosa National Park, would not exist today had it not been for the foresight of conservationists (e.g. ecologist and conservationist Dr. Daniel Janzen) to purchase cheap, abandoned pasture and let it regenerate naturally (United Nations Environment Programme 2003). Now, only a few decades later, these reserves act as important refuges of biodiversity (United Nations Environment Programme 2003). However, it is important to note that though secondary forest reserves help ease the pressure of habitat loss experienced by local species, secondary forest mostly benefits generalist species (species that can tolerate a wide range of environmental conditions and lifestyles), rather than those that specialize in "old growth" forest (Gardner *et al.* 2006). Fragments of secondary growth may regain much of their functionality relatively quickly, but to truly regenerate to the original "climax community" could take hundreds of years (Finegan 1996). From a conservation standpoint, it is important to understand the dynamics of how land naturally regenerates from anthropogenic disturbance.

Soil characteristics are one especially influential ecological force affecting forest regeneration (Janos 1983, Neale 1997, Smith and Read 1997). For example, the original land transformation or disturbance can significantly affect the kinds of plant communities that can exist on a site (Bazzaz and Pickett 1980). A site that has lost most of its topsoil and has become exceptionally nutrient poor may be colonized by some "weedy" species, but could remain too depleted of nutrients to ever succeed to the community that once inhabited it (Bazzaz and Pickett 1980).

Specifically, tropical rainforests are known for their biodiversity and high productivity (Terborgh 1992). However, a rainforest's fertility does not come from the richness of the soil, as most neotropical soils are shallow, nutrient-poor, acidic, and phosphorus-deficient (Janos 1983, Bazzaz and Pickett 1980). These soils consist primarily of weathered clay and are a product of constant rains and lack of geologically recent glaciations events (glaciers have not been in the tropics to scrape off the weathered topsoil, nor have they deposited loamy soil from other locations; Buol 2003). The frequent rainfalls of the tropics leach easily-dissolved minerals (like phosphorus) from the top soil layers, and carry them downstream, leaving large deposits of iron and aluminum oxides that are toxic to many plants (Buol 2003). Leaf litter is a critical source of nutrients for plant communities in areas with depleted soils; it is quickly decomposed and the nutrients either quickly absorbed by plants or leached out of the soil by a rainstorm (St. John *et al.*. 1983).

One factor that encourages a rainforest's luxurious growth is a very common mutualism with Arbuscular Mycorrhizae (AM) (Schubler *et al.* 2002). AM are a phylum (*Glomeromycota*) of fungi that penetrate the cortical cells of the roots of a host plant, and are distinguished by their formation of "tree-like" structures called arbuscules within the cortical cell of the roots of the

host plant (Schubler *et al.* 200, Janos 1983). Mutualisms with AM are one of the most frequent symbiotic plant adaptations to low-phosphorus soils (Smith *et al.* 2010); so common, in fact, that it is estimated that AM symbioses are formed by approximately 80% of all vascular plant species (Schubler *et al.* 2002). These fungi are obligate symbionts, meaning that they cannot live without the host plant's supply of energy-rich carbon compounds (Janos 1983). In return, mycorrhizae uptake inorganic phosphorus, as well as nitrogen and zinc, from the soil through their external hyphae for the host plant's use (Smith *et al.* 2010). AM can provide otherwise unavailable soil-derived nutrients to the host plant, increased absorption surface area, above ground productivity, and can even act to protect the host plant from pathogens and other environmental stressors (Jeffries *et al.* 2003).

Though the majority of tropical plants are able to associate with AM, the degree to which host plants are reliant upon mycorrhizae infection varies. Obligate mycotrophs completely depend on this mutualism, and may not be able to grow to maturity, or even germinate successfully without association with AM (Janos 1983, Johnson *et al.* 1991). Still, many other species are facultative mycotrophs, and associate with AM when conditions warrant, like in nutrient-depleted soils (Zangaro *et al.* 2003). Few tropical species are completely non-mycotrophic (the primary non-mycotrophic families are Brassicaceae, Cyperaceae, and Amaranthaceae; Howeler *et al.* 1987). An individual plant's competitive ability is strongly influenced by factors that affect its nutrient absorption, such as benefits derived from its association with mycorrhizae. Competition within the community for the same nutrients creates differential rates of growth and reproductive success, and this, in turn, can influence the composition of successional communities (Smith *et al.* 2010).

These initial conditions in nutrient levels and AM spore abundance may result from the type of land-use or the previous plant community prior to forest regeneration. For example, the farming of an obligately mycotrophic crop like *Lactuca sativa* (common lettuce) could maintain a high AM abundance in the soil (Janos 1983, Miller and Jackson 1998). Conversely, if the soil contains an excess of nutrients (such as from intensive fertilizer input), this could render AM obsolete (Janos 1983). In a case of high soil nutrient levels, host plants would do better to absorb the soil's abundant nutrients themselves, rather than trade away their photosynthetic products for AM to perform this function. Soil characteristics may also change over regeneration time in response to variations in the plant community. As a forest regenerates, decomposed leaf litter may accumulate in the soil and enrich the site with macronutrients (especially phosphorus) as the community gains biomass (Guariguata and Ostertag 2001). Also, soil should become less compact as new plant growth introduces structural complexity (Guariguata and Ostertag 2001). Thus, AM spore abundance and other edaphic characteristics like nutrient composition may work together to determine which plants can or cannot inhabit a regenerating area.

Much of the research concerning the role of mycorrhizae in tropical regeneration is largely speculative and sometimes contradictory. Janos (1980) theorized that nutrient-poor soils usually favor either obligatory mycotrophic species-dominant, or non-mycotrophic species-dominant communities. He argued that if AM are initially present in poor soils, obligatory mycotrophic species should be the most competitive. This would support a continuous abundance of AM spores and ensure the long-term dominance of mycotrophic species. However, if the soil is both nutrient-poor and lacking AM, it should favor non-mycotrophic species (which he claims are most pioneer species), which can out-compete mycotrophic species when AM are not present. Without a host, the AM (as well as the short-lived spores, which are

viable for about 4 under ideal conditions) would soon die, ensuring the continued dominance of non-mycotrophic species in the area (Janos 1980).

Building on this research, Rogers (1998) hypothesized that forest succession following disturbance is not determined as much by original soil characteristics as it is by the success of pioneer species. He suggested that the trajectory of succession depends on the creation of better growing conditions through these pioneer species, which alter the microclimate—they block the wind, decrease the temperature, block direct sunlight, and add detritus to the soil which increases the forest's humidity and water storage potential (Guariguata and Ostertag 2001). This provides a better habitat for understory and later-succession plants, as well for the primary dispersal agents of spores, such as burrowing vertebrates like moles and rabbits (Killham 1994, Kwan 1995, Wolf 1998). Rogers argued that mycorrhizal spore abundance in the soil does not affect pioneer communities as much as it does later successional stages.

If one combines Janos' and Rogers' predictions, one would expect to find (assuming pastures are nutrient poor and have few mycorrhizae) that spore number increases with regeneration time as the plot is colonized, first by non-mycotrophic pioneer species, which over time should attract mycorrhizal dispersal agents (e.g. rodents) and be colonized by later-successional mycotrophic species. These changes should also be reflected by other changes in the soil as the area regenerates from pastureland, such as decreasing bulk density, and increasing macro-nutrient concentrations.

In sharp contrast, however, is the work of Zangaro *et al.*(2003), demonstrating there is indeed disagreement within the field over how AM, soil, and plant communities affect each other as regeneration occurs. The authors presented data that early-seral species were more strongly colonized with AM, rather than the late-seral species Janos predicted (Zangaro *et al.* 2003).

Zangaro *et al.* (2003) argued that mycotrophism is essential in the initial re-colonization of a site following disturbance, and that it aids in seedling recruitment and the fast growth of pioneer species, especially in low-fertility soils. The authors claimed that AM are most abundant shortly after colonization by pioneer species, and that they decrease in abundance as pioneer species are replaced by less-mycotrophic, mid- and late-seral species (Zangaro *et al.* 2003). One explanation for this observed relationship is that pioneer species require association for rapid growth in low-phosphorus environments (such as in the neotropics). Pioneer species usually have small, wind dispersed seeds with few nutrient reserves (Zangaro *et al.* 2003). Large-seeded canopy trees, on the other hand, may be less reliant upon mycorrhizae, because they are slow-growing, and because they are able to utilize the nutrient stores in their seeds (Zangaro *et al.* 2003).

If one follows Zangaro *et al.*'s line of reasoning, assuming that pastures are nutrient poor, but that AM are already present in the soil (because most grasses are facultative mycotrophs), one might expect to find that spore number decreases with regeneration time. Mycotrophic pioneer species ideally would colonize the pasture and grow as rapidly as possible. As bulk density decreases, leaf detritus and macronutrients increase, and a short "canopy" is created. With appropriate conditions now established, later-successional species can colonize the area, which, requiring less mycorrhizae infection for nutrient absorption would roughly correspond to fewer AM spores in the soil.

As evidenced by the incongruity of predictions in the literature, it is not clear how a Neotropical soil's nutrient systems change with regeneration, or AM spore abundance. With the assumption that pastures are relatively similar in soil makeup and AM spore number, the present study examines spore abundance, nitrogen, phosphorus, potassium, pH, and bulk soil density for

plots of various stages of regeneration from pasture (ages 0-45, and primary forest, N=15) in the Premontane Wet Forest near San Luis, Costa Rica.

METHODS

I collected soil samples in the Premontane Neotropical Wet Forest (Holdridge life zone) near San Luis, Costa Rica (10.28290, -84.79857) on November 1st-15th, 2010, at an elevation of 1055 to 1200 m. The soils of the greater Monteverde area are primarily Oxisols and Ultisols (according to the USDA soil taxonomy), meaning they are weathered, clay soils, slightly acidic, and low in phosphorus. I first met with landowners in the San Luis area, as well as with staff members of the University of Georgia Research Station to determine each forest's age since disturbance and previous land use type. I selected 12 sites of varying regeneration time (0 to 45 years) from the pastures and secondary forests of San Luis and on the trails around the UGA campus. I also selected 3 additional sites of primary forest to compare with the pastureland and secondary growth (15 sites total). I defined primary forest in this study as old-growth forest for which the age is unknown and which is thought to be historically undisturbed by humans (excluding trails). Though primary forest sites lay "outside of the regeneration spectrum" because they have presumably never been disturbed, it seems reasonable to assume that if the soil composition follows some trend with increasing regeneration time, then the plots would eventually converge on the primary forests characteristics.

I removed three soil samples at each site with a soil borer (volume of 365 cm^3) to a depth of 15 cm, after brushing away surface debris and plant material. This depth is commonly used to capture the best representation of spore numbers (Johnson *et al.* 1991).

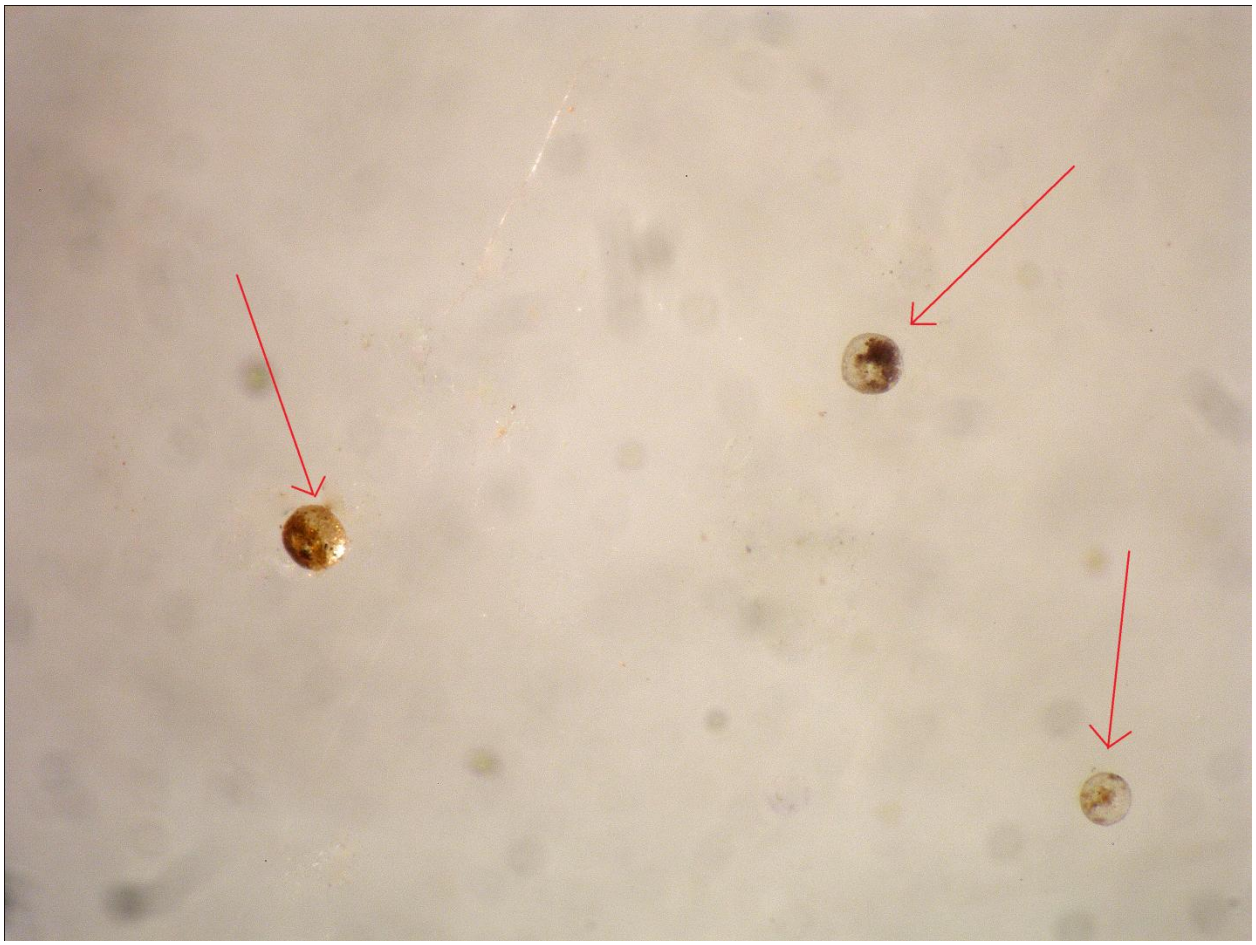

FIGURE 1. Three Arbuscular Mycorrhizae (AM) spores, (arrows), as seen under a dissecting microscope (magnification 40X). These spores were extracted from 7.5 g dry weight of soil from a regenerating pasture near San Luis, Costa Rica using a wet-sieving and centrifugation method.

All samples were taken from level areas with at least 15 cm of topsoil, and were collected at least 10 m or more from the nearest road, stream, trail, or other habitat type. Each sample was kept separate for individual analysis. Each soil sample was dried for 12 hours in a drying oven set to 63° C (145° F), and was then weighed to obtain the dry weight. This was divided by the soil borer's volume to determine each soil sample's bulk density (a rough measure of soil compaction). I then used a wet-sieving and centrifugation technique (adapted from Wolf 1998)

to separate the AM spores from 7.5 grams of each soil sample. I stirred each sample into 500 mL of water to dislodge spores and clumps of soil, and rinsed the solution through a series of nested sieves (250 µm and 125 µm). Therefore, I counted spores with a diameter of roughly 125-250 µm, which captures the midrange of most AM spores (Wolf 1998). I removed and distributed the sievate with water into 16 2mL plastic "bullet" centrifuge tubes, and centrifuged each soil sample at 3600 rpm for 45 seconds. I removed the supernatant from each centrifuge tube with a pipette, and examined it drop by drop on a Petri dish under a dissecting microscope to count the number of spores. The dried soil samples were then tested using a LaMotte soil testing kit for pH and for nitrate nitrogen (N), potassium (K), phosphorus (P), and pH.

RESULTS

AM Spore Abundance and Chemical Analysis

The number of spores in each 7.5 g subsample varied substantially within sites (mean spore number 54 ± Std. dev. 22, N = 15); therefore, I averaged AM spore numbers from each of the three separate soil samples for my calculations (see Table 1). The sites ranged from an average spore count of about 4 to 97 spores per 7.5 g soil, with the lowest from a pastureland site, and the highest found in the soil of 25 year old pastureland re-growth. Average spore abundance also varied substantially among the three separate pastureland and primary forest sites, respectively (the average spore count per 7.5 g subsample, in pastureland ranged from 4 to 83 spores per 7.5 g soil, and in primary forest from 33 to 71 spores per 7.5 g soil). Spore number was not significantly affected by regeneration age (regression, $p = 0.6382$, $R^2 = 0.0174$, N=12). A correlation table (Table 2) was constructed to determine how the physical and chemical soil characteristics were inter-related. pH was significantly correlated with regeneration time

(R=0.05, p<.05), but with the removal of a few apparent outliers, the trend was no longer significant (R=.42, p>.05). Also, K was positively correlated with bulk soil density, although I have reason to believe that the bulk density measure was misrepresentative of soil compaction (see Discussion).

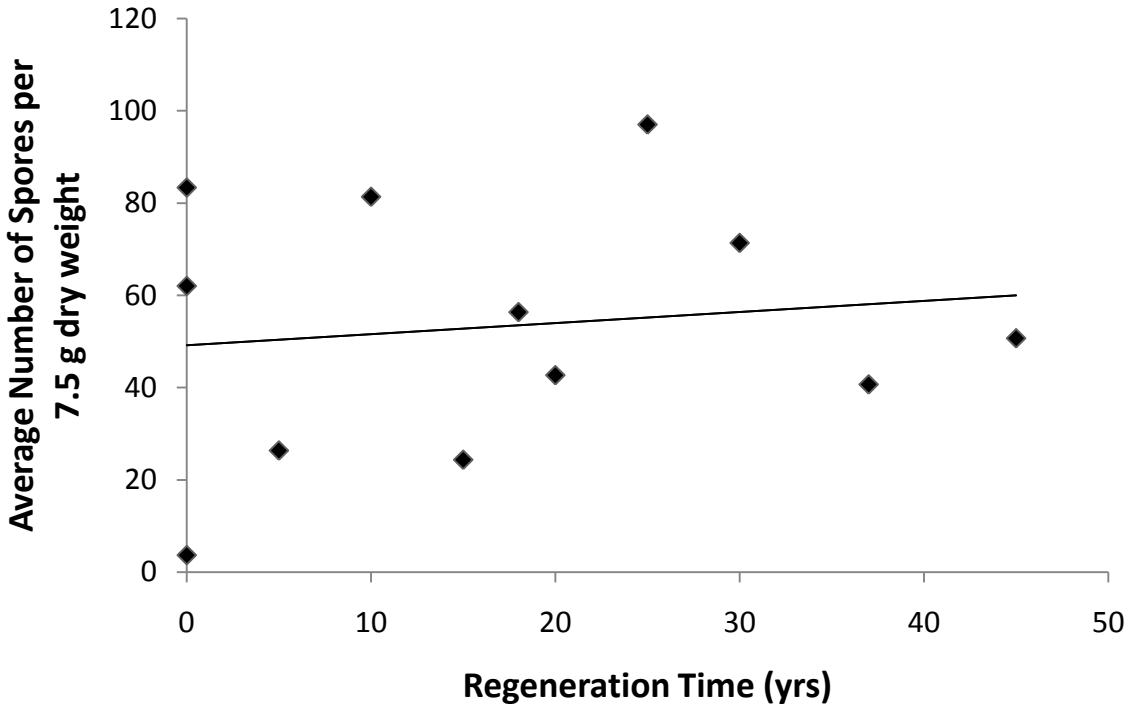

FIGURE 2. Number of average AM spores per 7.5 gram subsample with increasing natural regeneration time since pastureland. Spore number does not significantly increase with time ($p = 0.6832$, $R^2 = 0.0174$, N = 12, line of best fit: $y = 0.2406 x + 49.195$).

TABLE 1. Average soil characteristics of the 15 study sites of pasture, regenerating forest, or primary forest near San Luis, Costa Rica. N=3 for each average.

Site Age/ Type	Avg. Spore #	Avg. Bulk Soil Density (g/cm^3)	Avg. N (kg/ha)	Avg. P (kg/ha)	Avg. K (mL^{-1})*	Avg. pH
Pasture 1	4	0.50	16.81	106.48	0.44	5.43
Pasture 2	83	0.38	14.95	78.46	0.50	6.17
Pasture 3	62	0.59	46.70	117.69	0.65	5.83
5 years	26	0.62	26.15	74.73	0.59	6.27
10 years	81	0.48	44.84	37.36	0.54	6.30
15 years	24	0.41	18.68	102.75	0.47	6.67
18 years	56	0.44	59.78	186.82	0.66	6.30
20 years	43	0.40	63.52	46.70	0.47	6.23
25 years	97	0.32	13.08	140.11	0.43	6.53
30 years	71	0.53	44.84	121.43	0.54	6.30
37 years	41	0.45	37.36	95.28	0.59	6.40
45 years	51	0.58	39.23	84.07	0.83	6.33
Primary 1	33	0.33	18.68	168.13	0.44	6.50
Primary 2	72	0.44	44.84	84.07	0.62	6.10
Primary 3	66	0.38	44.84	186.82	0.64	6.10

***** The measurement for K (mL^{-1}) is an expression of relative abundance based on the soil testing procedure in the LaMotte Soil Testing Kit. This does not directly correlate to kg/ha of K at each site.

TABLE 2. Correlations among AM spore number, regeneration age, and the other soil properties of 12 pasture and secondary forest sites. Only site age with pH, and bulk density with K, were significantly correlated ($p < 0.05$). Spore abundance was not significantly correlated with any other soil characteristic.

	Spore #	Age	Bulk Density	N	P	K	pH
Spore #	--	--	--	--	--	--	--
Age	+ 0.13	--	--	--	--	--	--
Bulk Density	- 0.35	- 0.05	--	--	--	--	--
N	+ 0.06	+ 0.26	+ 0.24	--	--	--	--
P	+ 0.09	+ 0.10	- 0.16	- 0.01	--	--	--
K	+ 0.02	+ 0.43	+ 0.62*	+ 0.42	+ 0.11	--	--
pH	+ 0.36	+ 0.55*	- 0.37	+ 0.02	+ 0.01	+ 0.05	--

* indicates $p < 0.05$

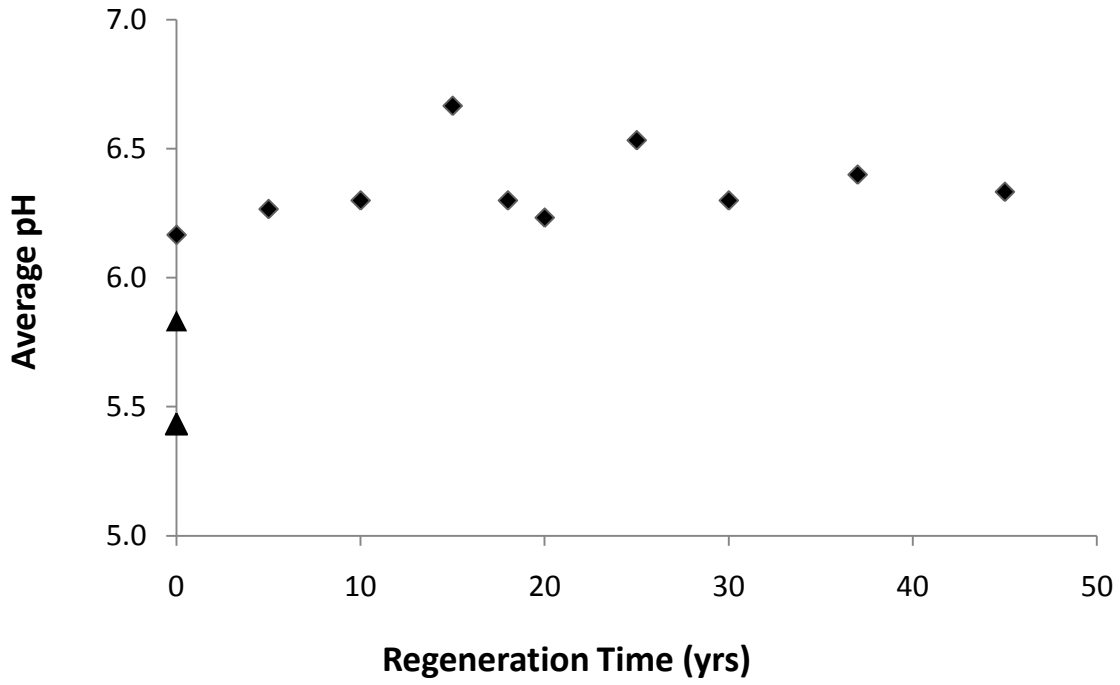

FIGURE 3. pH was significantly correlated with regeneration time ($R=0.55$, $p < 0.05$). However, with the removal of two possible outliers (triangles), this trend is no longer significant ($R=0.42$, $p > 0.05$).

Qualitative Observations

I observed a typical pattern of neotropical community succession (Zangaro *et al.* 2003, previous personal observations), with grass-dominated pasture first being colonized by ground-sprawling vines, small shrubs, herbaceous plants, Urticaceae, and early successional tree seedlings. This seemed to progress to dominance by *Heliconia* and vines while the seedlings increased in height. The understory continued to grow denser with Melastomataceae and Araceae species, and some understory palms. Finally, the older sites seemed to be dominated by large trees (and no Urticaceae), with lianas, Piperaceae, Melastomataceae, ferns and understory palms present. As best as I can tell from the literature, all of the species I observed are at least facultative mycotrophs, meaning they have the capacity to associate with AM, the extent of which may be influenced by current soil and climatic conditions (Howeler *et al.* 1987, Zangaro *et al.* 2003).

I also observed that pasture soil samples were muddy and compacted from being trampled by cows, only covered in a thin layer and roots of grass and little humus. Humus and leaf litter seemed to increase with regeneration time, and compaction seemed to decrease, though leveling out at a mid-regeneration time of approximately 25 years. This qualitative observation, therefore, contradicts my measure of compaction (bulk density) for which there was no trend with increasing regeneration age.

DISCUSSION

There was no observed trend between spore number and regeneration age, and it seems that soil compaction, N, P, K, and pH were not acting as confounding variables (they were not good predictors of spore abundance). Also, edaphic traits did not follow any clear trend with regeneration age. Though pH increased slightly with regeneration time (becoming more neutral),

this trend was not significant following the removal of two possible outliers. There is no agreement in the literature as to whether there are consistent trends in edaphic characteristics as a plot regenerates from disturbance. A review by Guariguata and Ostertag (2001) identified several seemingly contradictory studies; some that observed soils becoming enriched with regeneration, and some that observed soils becoming "depleted". They suggest that, ultimately, how nutrient content changes over time is a balance between the storage of nutrients in biomass, the rate of turnover (e.g. the creation of leaf litter) and decomposition of this biomass, and the subsequent leaching of nutrients from the soil (Guariguata and Ostertag 2001).

The process of regeneration may influence this nutrient balance—for example, rapidly-growing pioneer species may increase the plant cover of a disturbed site, which might eventually result in more leaf litter deposition. The new community may create favorable conditions for decomposers, so the leaf litter decomposes quickly, and the plants are able to utilize this source of nutrients before it gets washed away. This could be reflected by low levels of extractable nutrients in the soil, but rapid nutrient storage within the biomass of the plant community. However, if a regenerating area becomes sheltered with slow-growing late-seral species, this could increase the structural complexity of the soil by creating a matrix of roots, and possibly decrease the rate of leaching. In this case, decomposing leaf litter might accumulate, enriching the soil faster than the slow-growing species can absorb the nutrients, and resulting in a trend of soil enrichment with regeneration.

Along these lines, I qualitatively observed an increase in leaf litter and humus with regeneration age, and with it a decrease in soil compaction. However, this did not correspond to an increase in nutrient availability, and it was not represented by my measure for compaction (bulk soil density). The bulk soil density measurement may have been a flawed measure of soil

compaction, as an unusually sandy or heavy soil would have a high density but would not be indicative of soil compaction, possibly obfuscating the results.

Trends along the successional gradient would be difficult to discern because of high observed within-site and between-site variability in spore number. It cannot be excluded that edaphic trends do exist in the passive regeneration of pastureland, but that such trends were not observed due to differing initial spore or nutrient levels between the original pastureland of each site.

In terms of the hypotheses discussed in the introduction, the observations presented here support neither the hypothesis that spore numbers increased as mycotrophic species colonized the area, nor the hypothesis that spore numbers decreased with the eventual colonization of weakly-mycotrophic late-seral species. Ultimately, the edaphic characteristics of the soil were heterogeneous—both within one site, and among sites of similar regeneration age and disturbance type. Moreover, AM spores seemed to be distributed in a patchy or inconsistent manner (independent of N, P, K, pH, bulk density, and regeneration age).

It is debated in the literature whether mycotrophic host distribution causes this heterogeneity in spore abundance. Carvalho *et al.* (2003) found patchy AM spore distribution correlated with both proximity to host plants and to amount of organic matter. Alternatively, Friese and Koske (1991) found that spores were not significantly correlated with host plants or the organic content of the soil. However, these studies were conducted in Portuguese salt marshes and in a sand dune ecosystem in the United States, respectively, so the two are hardly comparable, especially for use in comparison with Premontane Moist Forest. However, it seems feasible for my results to have been confounded by my inability to track each soil sample's proximity to mycotrophic host plants.

Other possible explanations for the observed lack of trend between spore number and regeneration time are that the number of AM spores may not be a good indicator of the degree of host plant infection rate (Friese and Koske 1991, Johnson *et al.* 1991). Because infectivity indirectly accounts for all types of AM propagules (spores, hyphae, and AM roots), solely measuring spore abundance may be an inaccurate representation of AM infectivity and distribution in the soil. Therefore, assessing mycorrhizal root infection percentage, rather than spore abundance, could reveal a correlation with forest regeneration time (Johnson *et al.* 1991).

Regardless of the somewhat simplistic theories that attempt to describe the relationship between soil, plants, and AM (which were discussed in the introduction; Janos 1980, Rogers 1998, Zangaro *et al.* 2003), the heterogeneous distribution of AM spores is a common theme in the literature (Anderson *et al.* 1983; St. John *et al.* 1983; Facelli and Facelli 2002). This patchy distribution may have its own unique effects on the regenerating plant community. St. John *et al.* (1983) suggested that, because soil decomposition activity is heterogeneous, the resulting nutrient availability will also be heterogeneous in distribution (St. John *et al.* 1983). The authors argue for the "selective exploitation of localized nutrient-rich sites"—the idea that both plant roots and mycorrhizal hyphae grow out randomly from the plant, and, in the event they encounter a nutrient rich site, the root or hypha branches after this encounter (St. John *et al.* 1983). The individual plant's exploitation of chance proximity to a nutrient-rich micro-site provides it with a greater fitness. If some plants randomly achieve greater fitness due to change proximity to nutrient-rich sites, this could create heterogeneity in community structure, or "competitive asymmetry", which could therefore influence future succession (Facelli and Facelli 2002).

Finally, because of the widespread distribution of AM in the soils near San Luis, it is possible that they do little to create competition, and that most disturbed sites, though being nutrient depleted, are not inhibiting native mycotroph colonization because AM are fairly common in all soils. Despite the large amount of literature on the role of AM and edaphic characteristics in regeneration, it is, in practice, difficult to design an experiment that is comprehensive in accounting for the extensive factors that influence nutrient availability, AM infectivity, community succession, and spatial heterogeneity. Furthermore, because the dynamics of edaphic characters are so dependent on soil type, the source of disturbance, the type of forest, and the stage of regeneration, it is exceptionally difficult to compare the results of studies from different contexts (Guariguata and Ostertag 2001). Thus, it is not clear how AM and edaphic characteristics may respond to pasture regeneration.

The scope of my study was too small to elucidate the plant—soil—mycorrhizal relationship. I was limited to examining the relationship from one direction, namely how regenerational age (and therefore general changes in plant community) affects soil characteristics and AM spore abundance. However, the results of the present study underline the importance of understanding a site's spatial heterogeneity (in both AM spore inoculum potential and edaphic characteristics) before initiating a study of successional changes in soil composition. It also demonstrates the amount of uncertainty involved in choosing to study a regenerational chronosequence (a "snapshot" of the various sites at different stages of regeneration), rather than directly tracking each site over the course of its regenerational history. .

A long-term study is needed to assess the interaction of mycorrhizae and edaphic parameters along a successional gradient, after artificially creating identical "baseline" conditions for each pasture (e.g. each site's initial nutrient composition, or the heterogeneity of

AM inoculum), rather than assuming these starting conditions to be equivalent. Also, I did not have access to potentially important information, like how long each pasture had been established, or the mechanism by which it was originally deforested. A study with manually controlled plots would not only control for these variables, but it could also manipulate them (e.g. which regenerates faster? A pasture created by burning or by clear-cutting?). In a longer-term experiment, and ideally with abundant funding and labor at his or her disposal, the researcher could obtain a robust data set, as well as document seasonal and environmental variations (some studies, e.g. Carvalho *et al.* 2001, find that propagule abundance and AM colonization may exhibit significant temporal or seasonal variation).

Such a project would allow the researcher to thoroughly document the succession of each plant community, measuring the growth rate and species composition of each plot, as well as changes in edaphic characteristics (including a more appropriate measure for soil compaction, and a wider range of important parameters, such as magnesium, sodium, calcium, sulfur, organic matter, leaf litter, humus, cation exchange capacity, and mycorrhizal inoculum potential values; Titus *et al.* 2002). Having established the initial conditions of each site, the researcher could clarify the plant—soil—mycorrhizae relationship. A researcher could track how (and how quickly) succession occurs, how this affects the soil characteristics and AM inoculum potential, and how these in turn affect future successional communities. Importantly, the study could adopt a broader perspective of tropical regeneration, and would therefore account for other important drivers of succession, such as light gaps.

Additionally, with ample lab support, the researcher could measure AM infectivity in the roots of plants (a time-intensive process) as a more direct representation of the actual AM presence in the soil. A side benefit of sampling host plants directly for AM infection is gaining

site-specific knowledge about to what degree certain species are mycotrophic (e.g. Is this species obligately or facultatively mycotrophic? Under what conditions?). Such an experimental set-up would also allow a researcher to model various anthropogenic disturbances from which to regenerate vegetation.

The results from a comprehensive study of edaphic characteristics and AM fungi in tropical regeneration would be exceptionally useful to the recovery of sites that have undergone severe disturbance, including sites that have lost significant amounts of topsoil due to years of erosion from industrial agricultural practices (Howeler *et al.* 1987). Such degraded sites are abundant, but, without the effective replacement of AM populations (through inoculation), mycotrophic colonizers may have low recruitment rates, resulting in a perpetual field of weedy, non-mycotrophic species, a field that is of little use to conservation (Howeler *et al.* 1987).

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